TRAIL: a molecule with multiple receptors and control mechanisms

Thomas S Griffith* and David H Lynch†

Apoptosis research is benefiting from bioinformatic approaches to identify new components of the cell death machinery and novel cell death inducers/receptors. Over the past year, knowledge of the system involving TNF-related apoptosis-inducing ligand (TRAIL) and its receptors has increased via genomic database analysis to include four distinct receptors that interact with a single ligand. Currently, these molecules are of major interest due to their potential roles and application in cancer therapy.

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Current Opinion in Immunology 1998, 10:559–563

http://biomednet.com/elecref/0952791501000559

Abbreviations

DD	death domain
EST	expressed sequence tag
FADD	Fas-associated DD protein
FLICE	FADD-like IL-1β-converting enzyme
FLIP	FLICE-like inhibitory protein
TNF	tumor necrosis factor
TNF-R1	TNF receptor 1
TRADD	TNF-R1-associated DD protein
TRAIL	TNF-related apoptosis-inducing ligand
RT-PCR	reverse transcriptase polymerase chain reaction

Introduction

Although cellular death was first observed over 150 years ago during studies on amphibian metamorphosis (cited in [1]), it was not pursued with as much interest as other basic cellular processes because most scientists thought it was simply a degenerative phenomenon produced by injury. The idea that cell death occurred as a genetically controlled event in healthy animals did not gain wide acceptance until the early 1980s through studies in the nematode *Caenorhabditis elegans* [2,3]. Dramatic advances in apoptosis research have occurred recently, with the identification and characterization of numerous cell-death-inducing molecules and their cognate receptors as well as the dissection of the molecular components of the cell death machinery. Many of these advances stem from bioinformatic approaches using motifs derived from previously characterized molecules to search genetic databases for related proteins (cloning *in silico*?). TRAIL (TNF-related apoptosis-inducing ligand) and its receptors form one ligand/receptor system that has been recently identified primarily through database screening, which has generated a great deal of interest in the field of cell death and will be the focus of this review.

TRAIL and apoptosis

While searching an expressed sequence tag (EST) database using a conserved sequence contained in many TNF family members, Wiley *et al.* [4] identified an EST that was then used to clone the full-length cDNA for TRAIL, also known as Apo-2 ligand [5]. Like all but one member of this cytokine family, TRAIL is a type II membrane protein having an intracellular amino-terminal portion and its carboxyl terminus outside the cell. The extracellular domain of TRAIL is homologous to that of other family members; its highest amino acid identity is to Fas ligand (28%), while it has significant identity to TNF-α (23%), lymphotoxin-α (23%) and lymphotoxin-β (22%). Based on the crystal structure of TNF [6] it seemed likely that the most biologically active form of TRAIL would be a trimer; indeed studies with soluble TRAIL have found multimeric, or cross-linked, versions to be more effective at inducing apoptosis than monomeric TRAIL [4]. In addition, these early studies identified two other unique characteristics of TRAIL: first, TRAIL induces apoptotic cell death only in tumorigenic or transformed cells and not in normal cells [4]; second, in contrast to other members of the TNF family, whose expression is tightly regulated and only transiently expressed on activated cells, mRNA for TRAIL is detected in a wide range of tissues including peripheral blood lymphocytes, spleen, thymus, prostate, ovary, small intestine, colon and placenta; but not brain, liver or testis [4]. This broad expression of TRAIL suggested that the regulation of TRAIL-induced death was through restricted receptor expression.

The TRAIL receptor family

The search for a TRAIL receptor ended, or so it seemed, in early 1997 with the identification of DR4. Using the sequence of the death domain (DD) from the TNF receptor 1 (TNF-R1) to search an EST database, Pan *et al.* [7•] identified an EST clone that was subsequently used to clone the full-length cDNA for DR4 (hereafter referred to as TRAIL-R1). This protein, of 468 amino acids, has many of the characteristics of other death-inducing receptors in the TNF receptor family. The signal peptide sequence at the beginning of the molecule suggests that TRAIL-R1 is a type I membrane protein, containing two extracellular cysteine-rich pseudorepeats. In addition, the cytoplasmic domain of TRAIL-R1 contains a DD similar to that in TNF-R1, DR3, Fas and CAR1. Interestingly, northern blot analysis indicates that TRAIL-R1 mRNA is expressed in many of the same tissues as TRAIL mRNA.

Shortly after the identification of TRAIL-R1, several groups reported a second receptor for TRAIL. Pan *et al.* [8•] and Sheridan *et al.* [9•] searched an EST database using the sequence of the ligand-binding domain of

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Shortly after the identification of TRAIL-R1, several groups reported a second receptor for TRAIL. Pan *et al.* [8•] and Sheridan *et al.* [9•] searched an EST database using the sequence of the ligand-binding domain of

TRAIL-R1 to find DR5 (or TRAIL-R2). In contrast, Walczak *et al.* [10•] biochemically purified TRAIL-R2 from the surface of TRAIL-sensitive cells. TRAIL-R2 is a protein, of 411 amino acids, which is highly homologous to TRAIL-R1 (58% identity). TRAIL-R2 also is a type I transmembrane protein, with two extracellular cysteine-rich pseudorepeats and a cytoplasmic DD; its mRNA is expressed in most of the same tissues as TRAIL-R1 and TRAIL. The existence of two distinct apoptosis-inducing TRAIL receptors increased the complexity of the TRAIL/TRAIL-R system, primarily in regard to how some (i.e. untransformed) cells remain resistant to TRAIL-induced death, whereas others (i.e. tumor cells or virally infected fibroblasts) are sensitive.

The differences in sensitivity of normal, versus tumor, cells to TRAIL cytotoxicity may be in the form of two additional TRAIL receptors, TRAIL-R3 and TRAIL-R4. The ligand-binding domain of TRAIL-R1 was used to search EST databases; thus the 299-amino-acid TRAIL-R3 [11•] (or TRID/DcR1; [8•,9•]) was discovered. It is 58% and 54% identical to TRAIL-R1 and TRAIL-R2, respectively. Then the gene encoding the 386-amino-acid TRAIL-R4 [12•] (or DcR2; [13]) was identified from a cDNA library using a probe from the TRAIL-R3 ligand-binding domain; it is 58%, 57% and 70% identical to TRAIL-R1, -R2 and -R3 respectively. One interesting aspect of TRAIL-R3 and -R4 is that while their extracellular domains are similar to the other two receptors, their cytoplasmic domains differ remarkably. TRAIL-R4 contains only a partial DD and does not mediate apoptosis upon ligation [12•,13]. TRAIL-R3 is, perhaps, even more unique because it is devoid of any transmembrane or cytoplasmic residues and is glycosylphosphatidylinositol-linked to the cell surface [8•,9•,11•]. Northern blot analyses have demonstrated that the number of tissues constitutively expressing mRNA for TRAIL-R4 is nearly as great as with TRAIL-R1 and TRAIL-R2 [12•,13] but that the range of tissues expressing TRAIL-R3 mRNA is more limited [8•,9•,11•]. The genes for the four receptors are tightly clustered on human chromosome 8p21-22 [10•–12•], suggesting that they evolved relatively recently via gene duplication.

TRAIL receptor expression and sensitivity/resistance

Constitutive expression of TRAIL mRNA in a wide variety of tissue and cell types [4] suggests that regulation of apoptosis is mediated by restricted expression of the TRAIL receptors; however, as noted above, mRNA for the four TRAIL receptors is also expressed in a wide range of normal tissues; furthermore, recent studies have demonstrated that each of the four receptors is capable of binding TRAIL with comparable binding affinity (less than 1 nanomolar) [11•,12•]. Thus, the current hypothesis is that the nonsignaling receptors act as 'decoys' and that this is the chief mechanism determining whether a cell is resistant or sensitive to TRAIL-induced death [8•,9•,13].

Support for the 'decoy hypothesis' arose from experiments utilizing TRAIL-sensitive target cells transfected with either TRAIL-R3 or -R4, that resulted in a reduction in the amount of apoptotic cell death [8•,9•,13]. Rather surprisingly, though, TRAIL-R4 appears to be more effective than TRAIL-R3 in protecting target cells to TRAIL-induced death [12•]. While TRAIL-R3 and/or -R4 expression may indeed be a means of regulating TRAIL-mediated apoptosis, results from our laboratory analyzing TRAIL-R mRNA expression using RT-PCR in a panel of human tumor cell lines indicated no correlation between TRAIL resistance and TRAIL-R3/-R4 mRNA expression (Table 1). Several TRAIL-sensitive tumor lines tested positive for TRAIL-R3 and/or -R4 mRNA. Likewise, some of the TRAIL-resistant lines were negative for TRAIL-R3 and/or -R4 mRNA. Screening of over 60 additional human tumor cell lines (kindly supplied by the National Cancer Institute) did not demonstrate any obvious correlation between TRAIL-R mRNA expression and the level of sensitivity to TRAIL (TS Griffith, DH Lynch, unpublished data). Although it is clearly possible that

Table 1

TRAIL receptor expression in human tumor cell lines and sensitivity to TRAIL-induced apoptosis.

Cell type	Sensitive/ resistant*	Expression of mRNA for TRAIL-R (TR)			
		TR-1	TR-2	TR-3	TR-4
Melanoma					
WM 9	Sensitive	+	+	+	+
WM 35	Sensitive	–	+	–	–
WM 98-1	Sensitive	+	+	–	–
WM 164	Resistant	–	+	+	–
WM 793	Sensitive	–	+	+	–
WM 852	Resistant	+	+	–	+
WM 902	Resistant	–	+	–	–
WM 1158	Sensitive	+	+	+	–
WM 1205	Sensitive	+	+	+	–
WM 1341	Resistant	+	+	–	–
WM 1552	Sensitive	+	+	+	ND
WM 1791	Resistant	+	+	+	–
WM 1799	Sensitive	+	+	+	+
WM 3211	Resistant	–	+	–	–
Colon carcinoma					
HT 29	Resistant	+	+	–	+
SW 620	Resistant	+	+	+	+
HCT-15	Sensitive	+	+	–	+
COLO 205	Sensitive	+	+	+	+
Breast adenocarcinoma					
MDA 231	Sensitive	+	+	–	+
MCF7	Sensitive	+	+	+	–
Lung adenocarcinoma					
H2126	Sensitive	+	+	–	+
Others					
Daudi	Resistant	+	+	–	+
Jurkat	Sensitive	+	+	–	+
K562	Resistant	+	+	+	–
Raji	Resistant	+	+	–	–
HL-60	Resistant	+	+	+	+
PS-1	Sensitive	+	+	–	+
U937	Sensitive	+	+	+	ND

*Cell lines were considered sensitive if there was >20% cell death upon addition of 300 ng/ml leucine-zipper–TRAIL [10•]. ND, not done.

mRNA expression may not reflect cell-surface expression of these proteins, data recently obtained using monoclonal antibodies specific for TRAIL receptors appear to be concordant with the PCR analyses (TS Griffith, MZ Kubin, unpublished data).

An alternative hypothesis explaining the differential sensitivity of normal cells and tumor cells to TRAIL may revolve around the ability of TRAIL-R4 to provide intracellular antiapoptotic signals. Activation of the transcription factor NF-κB can prevent cells from undergoing TNF-induced cell death, probably by transcriptionally upregulating a gene, or group of genes, whose products are critical for providing resistance to apoptosis [14,15]. Since ligation of TRAIL-R4 also results in the activation of NF-κB [12•], the failure to activate these antiapoptotic proteins may make previously resistant tumor cells sensitive to TRAIL-induced death; however, ligation of TRAIL-R1 and -R2 also results in the activation of NF-κB [16,17], though still resulting in apoptotic cell death. Thus, such explanations cannot fully account for resistance to TRAIL-induced apoptosis.

Perhaps a more tenable hypothesis involves differential expression of intracellular inhibitors of the apoptotic process. Initial experiments examining the signaling of the death-inducing TRAIL receptors were based on their similarity to the well characterized death receptors Fas and TNF-R1. Activation of the cell death machinery through Fas or TNF-R1 requires the binding of Fas-associated DD protein (FADD) directly (as in Fas-induced death) or indirectly (as in TNF-R1-induced death mediated by TRADD [TNF-R1-associated DD protein] binding to FADD), to the DDs of these receptors [18]. This facilitates the binding and activation of caspase-8 to the receptor complex, which in turn activates other caspases and leads to cell death [18,19]. Thus, it was tested whether TRAIL-R1 and -R2 also associated with the adaptor proteins — namely FADD, TRADD and RIP (receptor-interacting protein) — utilized by Fas and TNF-R1 [7•–9•]. Results from experiments using cells transfected with these adaptor proteins demonstrated that neither TRAIL-R1 nor -R2 bound directly to these adaptor molecules. Additional examination of the TRAIL-R1 and -R2 signaling pathways indicated that expression of a dominant-negative form of FADD was unable to block apoptotic death; whereas the addition of the caspase inhibitors, cytokine response modifier A (CrmA) and carbobenzyloxy (z) VAD, inhibited apoptosis mediated by TRAIL-R1 and -R2 [7•–9•]. This suggested the association of an unknown adaptor that connected TRAIL-R1 and -R2 to the cell death machinery. Subsequent studies, though, reported contradictory findings by showing the direct binding of FADD and TRADD to these two TRAIL receptors and the inhibition of TRAIL-induced death with dominant-negative forms of either FADD or TRADD [10•,16,17]. The reason behind these discrepancies is not entirely clear, but they may be explained by differences in the relative expression levels of these molecules in the transfectants used in the experiments.

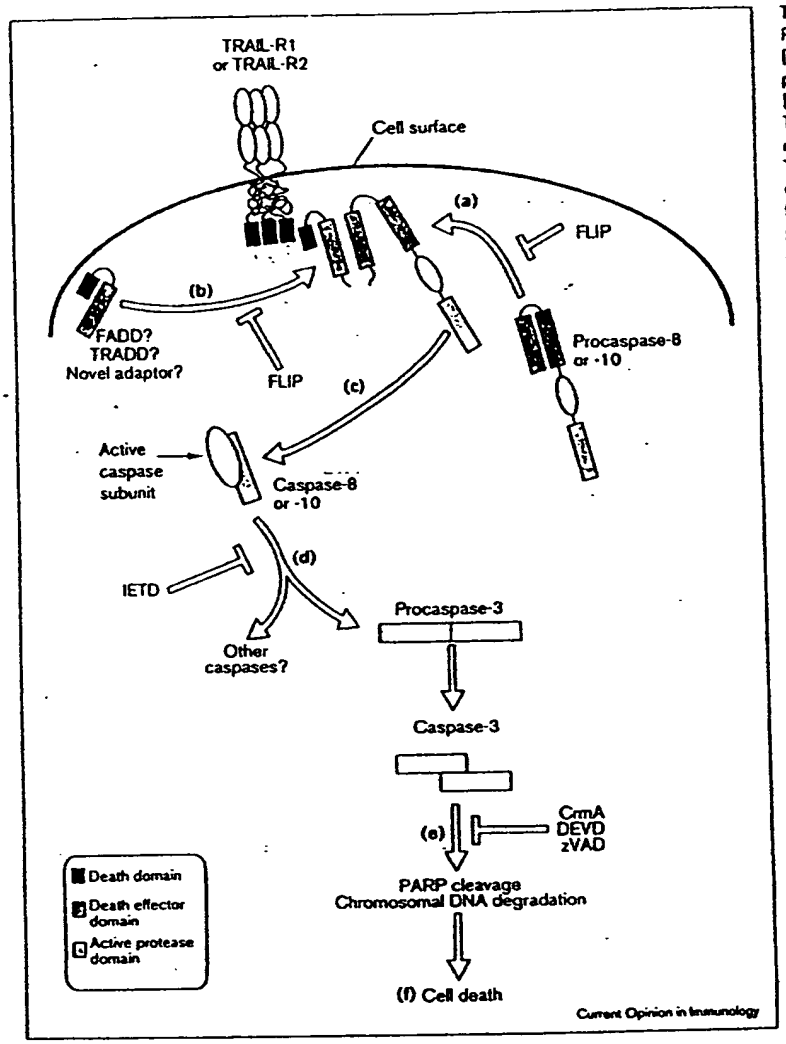
Studies from our laboratory examining caspase activation have revealed that many of the same caspases involved in Fas- and TNF-induced apoptosis are also important during TRAIL-induced death. Caspase-8 activation can be seen within minutes after the addition of TRAIL to sensitive cells, suggesting it may be one of the proximal components in the signaling pathway [20•]. Other reports have demonstrated the recruitment of caspase-10 (FLICE2/Mch4; [21,22]) to the TRAIL-R1 and -R2 signaling complexes [8•]. Shortly after caspase-8 activation we have also detected activation of caspase-3, one substrate of caspase-8 and -10 [20•]. Collectively, these results indicate the presence of a caspase pathway from the death-inducing TRAIL receptors that is similar to the one seen with other TNF family members, suggesting that many of the proteins recruited to other death receptor complexes are also recruited to TRAIL-R1 and -R2 complexes by way of specific adaptor molecules. This also leads to the potential of intracellular regulation of the caspase signaling cascade from the TRAIL receptors, as seen with other death receptors. A potential signaling cascade from TRAIL-R1 and -R2, as well as and the possible points of inhibition, are presented schematically in Figure 1.

Some degree of intracellular regulation is indicated when TRAIL-resistant melanoma cells are cultured in the presence of either actinomycin D or cycloheximide, resulting in their conversion to a TRAIL-sensitive phenotype [20•]. One possible explanation is the presence of an antiapoptotic molecule with a short half-life, that inhibits the caspase activation or mitochondrial alterations that are associated with induction of apoptosis [23,24]. When examining the levels of known apoptosis inhibitors, we found that TRAIL-resistant melanoma lines contained high levels of FADD-like IL-1β-converting enzyme (FLICE)-like inhibitory protein (FLIP) [20•] — a recently identified protein homologous to caspase-8 that lacks catalytic activity [25•]. FLIP levels decreased when cells were cultured with actinomycin D, paralleled by an increase in their sensitivity to the effects of TRAIL [20•]. While these results suggest a significant role for FLIP in controlling the susceptibility of tumor cells to the apoptosis-inducing effects of TRAIL, we cannot rule out the possibility that other factors, both intra- and extra-cellular, may also play roles in protecting normal cells from TPAIL-mediated apoptosis.

Conclusions

Although TRAIL potently induces apoptosis in tumor cells and some virally infected cells, it has little or no detectable cytotoxic effects on normal cells. Whereas this was first thought to be due to regulated expression of the TRAIL receptors, the fact that mRNA for both TRAIL and TRAIL receptors is often expressed in the same cells makes this explanation untenable. Indeed, the identification of four

Figure 1



The cell death signaling pathway from TRAIL-R1 or -R2, as suggested by [7•–10•,16–19,20•,21,22]. The inhibition points in this pathway are based on [7•–10•,16–19,20•,25•]. Upon activation of TRAIL-R1 or -R2, (a) procaspase-8 or -10 are recruited (b) with adaptor proteins (FADD, TRADD or a novel adaptor). FLIP can inhibit either step. (c) Active caspase-8 or -10 are formed and (d) activate procaspase-3 and possibly other caspases, which can be inhibited by IETD (single-letter code is used for amino acids). (e) Caspase-3 causes PARP (poly [ADP-ribose] polymerase) cleavage and DNA degradation (this can be blocked by cytokine response modifier [Crm]A, DEVD or carbobenzyloxy [z] VAD). (f) Ultimately cell death may occur.

distinct TRAIL receptors has significantly increased the potential complexity of this receptor/ligand system. Based on current information it seems likely that multiple factors, both intra- and extra-cellular, may function together to protect normal cells from the cytotoxic effects of TRAIL, but many questions remain. Are there additional receptors for TRAIL? Besides induction of apoptosis, are there other biological consequences of TRAIL ligation? What are the biological functions for TRAIL-R3 and -R4? Why should such a high proportion of tumor cells be sensitive to the apoptosis-inducing effects of TRAIL? And, perhaps most importantly, can we use the difference in sensitivity to the effects of TRAIL in tumor cells, compared to normal cells, to develop a powerful new therapeutic approach to the treatment of cancer *in vivo*?

Acknowledgements

The authors wish to thank Carl March, Ken Mohler and Michael Widmer for careful reading of the manuscript, Gary Carlton for preparation of the graphics and Anne C Aumell for her expert editorial assistance.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jacobson MD, Weil M, Raff MC: Programmed cell death in animal development. *Cell* 1997, 88:347-354

2. Horvitz HR, Shaham S, Hengartner MO: The genetics of programmed cell death in the nematode *Caenorhabditis elegans*. *Cold Spring Harb Symp Quant Biol* 1994, 59:377-385.

3. Ellis HM, Horvitz HR: Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 1986, 44:817-829.

4. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA, Goodwin RG: Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 1995, 3:673-682.

5. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A, Ashkenazi A: Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J Biol Chem* 1996, 271:12687-12690.

6. Banner DW, D'Arcy A, Janes W, Gentz R, Schoenfeld H-J, Broger C, Loetscher H, Lesslauer W: Crystal structure of the soluble human 55 kd TNF receptor-human TNFβ complex: implications for TNF receptor activation. *Cell* 1993, 73:431-445.

7. • Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM: The receptor for the cytotoxic ligand TRAIL. *Science* 1997, 276:111-113.

This describes the screening of an expressed sequence tag database with the death domain of TNF-receptor-1 permitted the cloning of the first of four TNF-related apoptosis-inducing ligand (TRAIL) receptor, DR4/TRAIL-R1.

8. • Pan G, Ni J, Wei Y-F, Yu GI, Gentz R, Dixit VM: An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* 1997, 277:815-818.

This provided bioinformatic identification of a second death-inducing TNF-related apoptosis-inducing ligand (TRAIL) receptor (DR5/TRAIL-R2) and increased the complexity of the TRAIL/TRAIL-receptor system.

9. • Sheridan JP, Marsters SA, Pitti PM, Gurney A, Skubatch M, Baldwin D, Ramakrishnan L, Gray CL, Baker K, Wood WI *et al.*: Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* 1997, 277:818-821.

This provided bioinformatic identification of a second death-inducing TNF-related apoptosis-inducing ligand (TRAIL) receptor (DR5/TRAIL-R2) and increased the complexity of the TRAIL/TRAIL-receptor system.

10. • Walczak H, Degli-Esposti MA, Johnson RS, Smolak PJ, Waugh JY, Boiani N, Timour MS, Gerhart MJ, Schooley KA, Smith CA *et al.*: TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. *EMBO J* 1997, 16:5386-5397.

This provided biochemical identification of a second death-inducing TNF-related apoptosis-inducing ligand (TRAIL) receptor (DR5/TRAIL-R2) and increased the complexity of the TRAIL/TRAIL-receptor system.

11. • Degli-Esposti MA, Smolak PJ, Walczak H, Waugh J, Huang C-P, DuBose RF, Goodwin RG, Smith CA: Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J Exp Med* 1997, 186:1165-1170.

As with [8•] and [9•], the ligand-binding domain of TNF-related apoptosis-inducing ligand (TRAIL)-R1 was used to identify a third, nonsignaling TRAIL-R (TRID/DcR1/TRAIL-R3), the existence of which possibly explains the differences in susceptibility between normal and tumor cells.

12. • Degli-Esposti MA, Dougall WC, Smolak PJ, Waugh JY, Smith CA, Goodwin RG: The novel receptor TRAIL-R4 induces NF-κB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. *Immunity* 1997, 7:813-820.

Using the ligand-binding domain of TNF-related apoptosis-inducing ligand receptor 3 (TRAIL-R3), a fourth TRAIL-R (TRAIL-R4) was identified that lacked a complete death domain but could signal for activation of the transcription factor NF-κB. This suggested resistance to TRAIL-induced death could result from TRAIL-R4, via an antiapoptotic signal (see [14] and [15]), in addition to the 'decoy' TRAIL-R3.

13. Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, Yuan J, Gurney A, Goddard AD, Godowski P, Ashkenazi A: A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr Biol* 1997, 7:1003-1006.

14. Beg AA, Baltimore D: An essential role for NF-κB in preventing TNF-α-induced cell death. *Science* 1996, 274:782-784.

15. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM: Suppression of TNF-α-induced apoptosis by NF-κB. *Science* 1996, 274:787-789.

16. Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L: Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-κB pathway. *Immunity* 1997, 7:821-830.

17. Schneider P, Thome M, Burns K, Bodmer J-L, Hofmann K, Kataoka T, Holler N, Tschopp J: TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-κB. *Immunity* 1997, 7:831-836.

18. Wallach D, Boldin M, Goncharov T, Goltsev Y, Mett I, Malinin N, Adar R, Kovalenko A, Varfolomeev E: Exploring cell death mechanisms by analyzing signaling cascades of the TNF/NGF receptor family. *Behring Inst Mitt* 1996, 97:144-155.

19. Chinnaiyan AM, Dixit VM: The cell-death machine. *Curr Biol* 1996, 6:555-562.

20. • Griffith TS, Chin WA, Jackson GC, Lynch DH, Kubin MZ: Intracellular regulation of TRAIL-induced apoptosis in human melanoma cells. *J Immunol* 1998, 161:in press.

This is a demonstration that sensitivity/resistance of tumor cells to TNF-related apoptosis-inducing ligand (TRAIL) does not correlate with TRAIL-receptor-3 or TRAIL-receptor-4 mRNA expression, but instead correlates with intracellular concentrations of Fas-associated death-domain-like IL-1β-converting enzyme (FLICE)-like inhibitory protein.

21. Vincenz C, Dixit VM: Fas-associated death domain protein interleukin-1β-converting enzyme 2 (FLICE2), an ICE/Ced-3 homologue, is proximally involved in CD95- and p55-mediated death signaling. *J Biol Chem* 1997, 272:6578-6583.

22. Fernandes-Alnemri T, Armstrong RC, Krebs J, Srinivasula SM, Wang L, Bullrich F, Fritz LC, Trapani JA, Tomaselli KJ, Litwick G, Alnemri ES: *In vitro* activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proc Natl Acad Sci USA* 1996, 93:7464-7469.

23. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD: The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 1997, 275:1132-1136.

24. Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT, Thompson CB: Bcl-x_L regulates the membrane potential and volume homeostasis of mitochondria. *Cell* 1997, 91:627-637.

25. • Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer J-L, Schroter M, Burns K, Mattmann C *et al.*: Inhibition of death receptor signals by cellular FLIP. *Nature* 1997, 388:190-195.

Regulation of death induction by members of the TNF receptor family was found to occur through the interaction of the caspase-8 homolog cFLIP with Fas-associated death domain and caspase-8.